

RECEIVED

OCT 01 1997

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

OCT - 2 1997

MERCHANT & GOULD
MINNEAPOLIS, MN 5540

To:

BRUESS, Steven C.
MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

19 SEP 1997

Applicant's or agent's file reference

600.311WOI1 IPD

IMPORTANT NOTIFICATION

International application No.

PCT/US 96/ 10252

International filing date (day/month/year)

07/06/1996

Priority date (day/month/year)

07/06/1995

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**
The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

Peter Ehrenreich

Telephone No.

D-


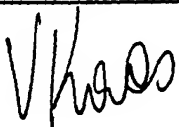
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 600.311WOI1	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US 96/ 10252	International filing date (day/month/year) 07/06/1996	Priority date (day/month/year) 07/06/1995
International Patent Classification (IPC) or national classification and IPC C12N15/31		
Applicant REGENTS OF THE UNIVERSITY OF MINNESOTA et al.		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>6</u> sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consists of a total of <u>6</u> sheets.</p>
<p>3. This report contains indications and corresponding pages relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input checked="" type="checkbox"/> Certain observations on the international application</p>

Date of submission of the demand 03/01/1997	Date of completion of this report 19 SEP 1997
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer  Telephone No.

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

☐ the international application as originally filed.

☒ the description, pages 1, 2, 4-80 _____, as originally filed,
pages _____, filed with the demand,
pages 3, 3a _____, filed with the letter of 01/08/97,
pages _____, filed with the letter of _____,

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-23 _____, filed with the letter of 01/08/97,
Nos. _____, filed with the letter of _____,

☒ the drawings, sheets/fig 1/9-9/9 _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____.
☐ the claims, Nos. _____.
☐ the drawings, sheets/fig _____.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-15, 18, 19, 22, 23_____	YES
	Claims 16, 17, 20, 21_____	NO
Inventive Step (IS)	Claims _____	YES
	Claims 1-23_____	NO
Industrial Applicability (IA)	Claims 1-13, 16-21_____	YES
	Claims 14, 15, 22, 23_____	NO

2. CITATIONS AND EXPLANATIONS

1) Reference is made to the following document:

D1 : International Immunology, vol. 5, no. 8, pages
869-875

2) The present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1, 9, 12, 13 does not involve an inventive step in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

D1 discloses the preparation of 9 mutants of SPE-A, each with a single amino-acid substitution, and the preparation of a mutant created by deletion of the 10 N-terminal amino acids of SPE-A ("SPEA(-10)"). Several mutations are disclosed to lead to a loss of function. The mutants are expressed as fusion proteins (see "Methods on page 870). D1 also discloses the injection of one of the mutants into mice. It is disclosed that the serum of these mice is capable of specifically inhibiting the

mitogenicity of SPE-A (see page 874, left hand column, second paragraph).

Although novel over D1, the mutations recited in claim 1 cannot be considered to represent a selection which involve an inventive step in that it appears from page 21, lines 16-25 of the present description that they are equivalent at least to mutations made in domain B-beta strands 4 and 5 as disclosed in D1 (see Ala-77, Ala-100 and Ala-104). This also applies to the corresponding nucleotide sequence and host cell of respectively claims 12 and 13.

Given that the mutations disclosed in D1 can also result in the loss of mitogenicity for T-cells (see page 873), the subject-matter of claim 9 also lacks inventiveness.

- 3) It appears that the single substitutions listed by dependent claims 2-8 do not lead to any new and unexpected effects in comparison to that observed with the single substitutions carried out in D1. Claims 2-8 are therefore not considered to involve an inventive step over D1. The said claims thus do not satisfy the criteria set forth in Article 33(3) PCT.
- 4) As acknowledged by the Applicant on pages 1-3, the role of SPE-A in toxic shock is well established in the art. The use of mutants equivalent to those disclosed in D1 as a vaccine to reduce symptoms associated with toxic shock is therefore obvious for the skilled person.

Claims 10, 11, 14 and 15 therefore do not satisfy the criteria set forth in Article 33(3) PCT.

- 5) The mutant SPE-A toxin of Claims 16 and 17 lack novelty over mutant "SPEA(-10)" disclosed D1. The latter is ob-

tained by deletion of the 10 N-terminal amino acids of SPE-A and can thus be considered to have more than one amino acid "changes" when compared to SPE-A. The lack of mitogenic activity of SPEA(-10) on T-cells is also disclosed in D1 (see page 873, left hand column). This also applies to the corresponding nucleotide sequence and host cell of respectively claims 20 and 21.

- 6) Again, as acknowledged by the Applicant on pages 1-3, the role of SPE-A in toxic shock is well established in the art. The use of mutant "SPE-A(-10)" known from D1 as a vaccine to reduce symptoms associated with toxic shock is therefore obvious for the skilled person. Moreover, such use is explicitly suggested on page 869 (last paragraph) of D1.

Claims 18, 19, 22 and 23 therefore lack an inventive step.

- 7) The subject-matter of claims 1-13 and 16-21 is susceptible of industrial applicability as defined in Article 33(4) PCT.
- 8) For the assessment of present claims 14, 15, 22 and 23 on the question as to whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

- 1) The expression "fragment thereof" in claim 1 can be interpreted in so many ways that it renders the scope of the claim unclear, contrary to Article 6 PCT.
- 2) As regards the function of the claimed mutant, the expression "substantially nonlethal" used in claims 1 and 16 is vague in that it does not specify any precise relation dose/mortality (Article 6 PCT).

staphylococcal toxic shock syndrome toxin 1,
staphylococcal enterotoxins A, B, Cn, D, E, G and H,
and non-group A streptococcal pyrogenic exotoxins.

- 5 These toxins have similar biochemical properties,
biological activities and various degrees of sequence
similarity.

The most severe manifestations of STSS are
hypotension and shock, that lead to death. It is
10 generally believed that leakage of fluid from the
intravascular to the interstitial space is the final
cause of hypotension, supported by the observation that
fluid replacement therapy is successful in preventing
shock in the rabbit model of STSS described above. It
15 has been hypothesized that SPE-A may act in several ways
on the host to induce this pathology. Certain single
amino acid substitutions in central regions of the SPE-A
molecule have been shown to affect the mitogenic
activity of and binding to a HLA class II molecules by
20 SPE-A (Hartwig et al. International Immunology 5:5, 869-
875 (1993)).

SPE-A has been shown to block liver clearance of
endotoxin of endogenous flora's origin, by comprising
the activity of liver Kupffer cells. This appears to
25 cause a significant increase in circulating endotoxin,
that through binding to lipopolysaccharide binding
protein (LBP) and CD14 signaling leads to macrophage
activation with subsequent release of TNF- α and other
cytokines. Support for the role of endotoxin in the
30 disease is given by the finding that the lethal effects
of SPE-A can be at least partially neutralized by the

administration to animals of polymyxin B or by the use of pathogen free rabbits.

Another modality of induction of shock could be the
5 direct activity of the toxin on capillary endothelial cells. This hypothesis stems from the finding that the staphylococcal pyrogenic toxin TSST-1 binds directly to the human umbilical cord vein cells and is cytotoxic to isolated porcine aortic endothelial cells.

WHAT IS CLAIMED IS:

1. A mutant SPE-A toxin or fragment thereof, the mutant SPE-A toxin comprising one to six amino acid substitutions and being substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin;

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

2. The mutant SPE-A toxin of claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

3. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic acid.

4. The mutant SPE-A toxin of claim 3, wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

5. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

5

6. The mutant SPE-A toxin of claim 5, wherein the substitution is asparagine-20 to aspartic acid.

10

7. The mutant SPE-A toxin of claim 5, further comprising substitution of cysteine-98, or lysine-157.

15

8. The mutant SPE-A toxin of claim 7, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

20

9. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A toxin.

25

10. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 1.

30

11. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

5 12. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

10 13. A stably transformed host cell comprising a DNA sequence according to claim 12.

14. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 10 to an animal.

15 15. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 10 to an animal.

20 16. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least two amino acid changes and is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

25 17. The mutant SPE-A toxin of claim 16, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is
30 not lethal, or the mutant is nonlethal but retains

mitogenicity comparable to that of the wild type SPE-A toxin.

18. A vaccine for protecting animals
5 against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 16.

19. A pharmaceutical composition
10 comprising: a mutant SPE-A according to claim 16 in admixture with a physiologically acceptable carrier.

20. A DNA sequence encoding a mutant SPE-A toxin according to claim 16.
15

21. A stably transformed host cell comprising a DNA sequence according to claim 29.

22. A method for protecting an animal
20 against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 18 to an animal.

23. A method for reducing symptoms
25 associated with toxic shock comprising: administering a vaccine according to claim 18 to an animal.

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.
Application Serial No : PCT/US96/10252
Filed : 07 June 1996
Agent Ref. : 600.311 WO11
Title : MUTANTS OF STREPTOCOCCAL TOXIN A
AND METHODS OF USE

RESPONSE TO WRITTEN OPINION

European Patent Office
D-80298 Munchen
GERMANY

Dear Sir:

In response to the written opinion mailed June 3, 1997, Applicant requests the following amendments be made to the patent application identified above.

IN THE SPECIFICATION

Please find enclosed replacement pages 3 and 3a which have been amended to refer to document D1 as prior art.

IN THE CLAIMS

Please amend the claims by substituting previous claim pages 81-83 with new claim pages 81-84. Amended claims 1-16 are replaced by amended claims 1-23. Amended claims 1-23 correspond to the original claims as follows:

<u>New Claim</u>	<u>Original Claim</u>
1	1, 5
2	1, 4, 5
3-8	4, 5
9	2, 3, 5
10	6
11	15
12	19
13	20

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.
 Application Serial No : PCT/US96/10252
 Filed : 07 June 1996
 Agent Ref. : 600.311WO11
 Title : MUTANTS OF STREPTOCOCCAL TOXIN A
 AND METHODS OF USE

14	22
15	22
16	1
17	2, 3, 5
18	6
19	15
20	19
21	20
22	22
23	22
canceled	7-14, 16-18, 21, 23-39

REMARKS

V. Reasoned Statement

2.2) Novelty

The Examiner asserted that claims 1 and 10-15 are not new with respect to cited document D1.

Amended claim 1 includes the limitations of claim 2, which was found to be new with respect to the cited reference. Therefore, amended claim 1 is new with respect to the cited document D1.

Independent claim 16 includes the limitation that the mutant toxin include at least two amino acid substitutions. Document D1 describes only single mutants. Therefore, claim 16 is new with respect to cited document D1.

2.3) and 2.4) Inventive Step

The Examiner asserted that claim 16 lacks an inventive step over cited document D1. The Examiner bases this rejection on the contention that the method of claim 16 includes

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.
Application Serial No : PCT/US96/10252
Filed : 07 June 1996
Agent Ref. : 600.311WOI1
Title : MUTANTS OF STREPTOCOCCAL TOXIN A
AND METHODS OF USE

administering mutants disclosed in document D1. Amended claim 1 includes the limitations of previous claim 2 and does not include mutants disclosed in document D1. The claimed mutants are neither disclosed nor are their properties or usefulness suggested by document D1. Therefore, document D1 does not disclose or make obvious the claimed method and claim 16 has an inventive step with respect to this document. Claim 1 is limited to mutant toxins with at least 2 amino acid changes, which excludes the mutants disclosed in document D1, and, as explained below, includes mutants with properties unexpected in light of the disclosure of document D1.

The Examiner asserts that claims 2-9 do not involve an inventive step over document D1. The Examiner bases this rejection on the contention that the claimed multiple mutants lack new and unexpected effects compared to the single mutants disclosed in D1.

Actually, the present application teaches several effects that are both new and unexpected in light of the disclosure of document D1. Document D1 describes only HLA class II binding by and mitogenic activity of mutant SPE-As. In contrast, the present application describes several important characteristics of SPE-A mutants: decreased mitogenicity for T-cells, reduced ability to cause endotoxin shock or lethality, increased or more consistent antigenicity, and protection from pyrogenicity or fever response. With respect to double mutants, the present application describes that single and double mutants unexpectedly differ in the protection they afford from fever and the enhancement phenomenon (the paragraph abridging pages 69-70 of the application as filed).

Furthermore, document D1 does not disclose aspects of a mutant that might indicate that the mutant is non-lethal and useful in a vaccine. No toxicity testing is disclosed in document D1. Neither does the MHC class II binding disclosed does not aid in production of a mutant that is useful as a vaccine. No vaccination is attempted. Document D1 does not disclose the effect of inoculation with mutant SPE-A on pyrogenicity, lethality, or other effects of wild-type SPE-A. Document D1 describes protein sequences, not useful mutants with one or more amino acid substitutions.

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.
 Application Serial No : PCT/US96/10252
 Filed : 07 June 1996
 Agent Ref. : 600.311WO11
 Title : MUTANTS OF STREPTOCOCCAL TOXIN A
 AND METHODS OF USE

Contrary to the Examiner's assertion, multiple mutations do lead to new and unexpected effects. Therefore, the invention as described in the amended claims includes an inventive step compared to cited document D1.

VII. Certain Defects

The Examiner indicated that document D1 should be identified in the description. Accompanying this response please find replacement pages 3 and 3a which include a brief discussion of document D1.

VIII. Certain Observations

1) Support

The Examiner asserted that claim 1 is not sufficiently supported in the description. Amended claim 1 recites mutant SPE-A molecules that enjoy substantial support in the description, in particular in Example 3 at pages 52-57 of the description as filed. The multiple mutants of claim 16 are supported throughout the specification, in particular in Examples 3-7 at pages 52-70 and at pages 25-26. Therefore, Applicant respectfully submits that amended claims 1 and 16 enjoy substantial support in the description over their entire scope.

2) Clarity

The Examiner objected to the use in claim 1 of the term "fragment thereof". This term is well defined in the application as filed. Fragments of SPE-A mutants are described in the application as filed at least at page 5, lines 17-26, and at page 28, line 16 through page 30. It is well known in that toxins such as SPE-A have structural domains, which are described in the present application at least at pages 21-24 and in Example 3 at pages 52-57. Fragments typically retain any structural domains needed for a desired function, have properties and activities described in the cited passages of the application, and have, for example, about 50

In re application of : REGENTS OF THE UNIVERSITY OF MINNESOTA et al.
Application Serial No : PCT/US96/10252
Filed : 07 June 1996
Agent Ref. : 600.311WOI1
Title : MUTANTS OF STREPTOCOCCAL TOXIN A
AND METHODS OF USE

amino acid residues. Based on the description in the application of the composition and properties of fragments of SPE-A, Applicants respectfully submit that SPE-A fragments are well defined in the specification and clearly recited in claim 1.

The Examiner objected to the use in claim 1 of the term "substantially nonlethal". The term substantially nonlethal is defined in the application at least at page 16, lines 11-20. According to this definition, a mutant SPE-A is substantially nonlethal if, when administered to a rabbit at the same dose as the wild type toxin, less than about 10-20% of the rabbits die. Accordingly, it is respectfully submitted that the term substantially nonlethal is well defined in the specification and is clear as recited in claim 1.

Consideration of these amendments and remarks is respectfully requested, and issuance of a favorable opinion is earnestly solicited.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT, P.A.

3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
United States of America
(612) 371-5240

Dated: August 1, 1997

By Mark T. Skoog
Mark T. Skoog
Reg. No. 33,924

staphylococcal toxic shock syndrome toxin 1,
staphylococcal enterotoxins A, B, Cn, D, E, G and H,
and non-group A streptococcal pyrogenic exotoxins.

- 5 These toxins have similar biochemical properties,
biological activities and various degrees of sequence
similarity.

The most severe manifestations of STSS are
hypotension and shock, that lead to death. It is
10 generally believed that leakage of fluid from the
intravascular to the interstitial space is the final
cause of hypotension, supported by the observation that
fluid replacement therapy is successful in preventing
shock in the rabbit model of STSS described above. It
15 has been hypothesized that SPE-A may act in several ways
on the host to induce this pathology. Certain single
amino acid substitutions in central regions of the SPE-A
molecule have been shown to affect the mitogenic
activity of and binding to a HLA class II molecules by
20 SPE-A (Hartwig et al. International Immunology 5:5, 869-
875 (1993)).

SPE-A has been shown to block liver clearance of
endotoxin of endogenous flora's origin, by comprising
the activity of liver Kupffer cells. This appears to
25 cause a significant increase in circulating endotoxin,
that through binding to lipopolysaccharide binding
protein (LBP) and CD14 signaling leads to macrophage
activation with subsequent release of TNF- α and other
cytokines. Support for the role of endotoxin in the
30 disease is given by the finding that the lethal effects
of SPE-A can be at least partially neutralized by the

administration to animals of polymyxin B or by the use of pathogen free rabbits.

Another modality of induction of shock could be the
5 direct activity of the toxin on capillary endothelial cells. This hypothesis stems from the finding that the staphylococcal pyrogenic toxin TSST-1 binds directly to the human umbilical cord vein cells and is cytotoxic to isolated porcine aortic endothelial cells.

WHAT IS CLAIMED IS:

1. A mutant SPE-A toxin or fragment thereof, the mutant SPE-A toxin comprising one to six
5 amino acid substitutions and being substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin;

wherein at least one of the substituted amino acids is positioned in N-terminal alpha helix 3, in
10 domain B beta strand 1, in domain B beta strand 2, in domain B beta strand 3, in domain A beta strand 6, in domain A beta strand 8, in domain A beta strand 9, in domain A beta strand 10, or is a cysteine.

15 2. The mutant SPE-A toxin of claim 1, wherein the mutant SPE-A toxin comprises one to six amino acid substitutions; and

wherein at least one of the substituted amino acids is asparagine-20, lysine-157, or cysteine-98.

20

3. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises the substitution of asparagine-20 to aspartic acid, glutamic acid, lysine or arginine; the
25 substitution of cysteine 98 to serine, alanine, glycine, or threonine; or the substitution of lysine-157 to glutamic acid or aspartic acid.

4. The mutant SPE-A toxin of claim 3,
30 wherein the at least one amino acid substitution comprises asparagine-20 to aspartic acid, cysteine 98 to serine, or lysine-157 to glutamic acid.

5. The mutant SPE-A toxin of claim 2, wherein the at least one amino acid substitution comprises substitution of asparagine-20.

5

6. The mutant SPE-A toxin of claim 5, wherein the substitution is asparagine-20 to aspartic acid.

10

7. The mutant SPE-A toxin of claim 5, further comprising substitution of cysteine-98, or lysine-157.

15

8. The mutant SPE-A toxin of claim 7, wherein the substitution is cysteine 98 to serine, or lysine-157 to glutamic acid.

20

9. The mutant SPE-A toxin of claim 1, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains mitogenicity comparable to that of the wild type SPE-A

25

toxin.

10. A vaccine for protecting animals against at least one biological activity of wild-type SPE-A comprising: an effective amount of at least one mutant SPE-A toxin according to claim 1.

30

11. A pharmaceutical composition comprising: a mutant SPE-A according to claim 1 in admixture with a physiologically acceptable carrier.

5 12. A DNA sequence encoding a mutant SPE-A toxin according to claim 1.

13. A stably transformed host cell comprising a DNA sequence according to claim 12.

10

14. A method for protecting an animal against at least one biological activity of a wild type SPE-A comprising: administering a vaccine according to claim 10 to an animal.

15

15. A method for reducing symptoms associated with toxic shock comprising: administering a vaccine according to claim 10 to an animal.

20

16. A mutant SPE-A toxin or fragment thereof, wherein the mutant has at least two amino acid changes and is substantially nonlethal compared with a protein substantially corresponding to wild type SPE-A toxin.

25

17. The mutant SPE-A toxin of claim 16, wherein the mutant has at least one of the following characteristics: the mutant has a decrease in mitogenicity for T-cells, the mutant does not
30 substantially enhance endotoxin shock, the mutant is not lethal, or the mutant is nonlethal but retains

mitogenicity comparable to that of the wild type SPE-A toxin.

18. A vaccine for protecting animals
5 against at least one biological activity of wild-type
SPE-A comprising: an effective amount of at least one
mutant SPE-A toxin according to claim 16.

19. A pharmaceutical composition
10 comprising: a mutant SPE-A according to claim 16 in
admixture with a physiologically acceptable carrier.

20. A DNA sequence encoding a mutant SPE-A
toxin according to claim 16.
15

21. A stably transformed host cell
comprising a DNA sequence according to claim 29.

22. A method for protecting an animal
20 against at least one biological activity of a wild
type SPE-A comprising: administering a vaccine
according to claim 18 to an animal.

23. A method for reducing symptoms
25 associated with toxic shock comprising: administering
a vaccine according to claim 18 to an animal.

PATENT COOPERATION TREATY

RECEIVED

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

JUN 09 1997

MERCHANT & GOULD
MINNEAPOLIS, MN 55402

To:

BRUESS, Steven C.
MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
ETATS-UNIS D'AMERIQUE

FFD REC'D

WRITTEN OPINION

JUN - 9 1997

(PCT Rule 66)

Atty-Response
3 Aug 97

Date of mailing
(day/month/year)

03 JUN 1997

Applicant's or agent's file reference
600.311WOI1

REPLY DUE

within **2** months/~~days~~
from the above date of mailing

International application No.

PCT/US 96/ 10252

International filing date (day/month/year)

07/06/1996

Priority date (day/month/year)

07/06/1995

International Patent Classification (IPC) or both national classification and IPC

C12N15/31

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA et al.

1. This written opinion is the first (first, etc.) drawn up by this International Preliminary Examining Authority.

2. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 07/10/1997

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

Examiner

V. Kaas
V. Kaas

Formalities officer
(incl. extension of time limits)
Telephone No.

Peter Ehrenreich
Peter Ehrenreich

WRITTEN OPINION

Intern. application No.

PCT/US96/10252

I. Basis of the opinion

1. This opinion has been drawn up on the basis of (Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".):

☐ the international application as originally filed.

☒ the description, pages 1-80, 80a-80d _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,

☒ the claims, Nos. _____, as originally filed,
Nos. 1-16 _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,

☒ the drawings, sheets/fig 1/9-9/9 _____, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____.
☐ the claims, Nos. _____.
☐ the drawings, sheets/fig _____.

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

WRITTEN OPINION

Intern. application No.
PCT/US96/10252

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1, 10-15_____
	Claims _____
Inventive Step (IS)	Claims 1-16_____
	Claims _____
Industrial Applicability (IA)	Claims 15, 16 (no assessment)_____
	Claims _____

2. CITATIONS AND EXPLANATIONS

- 1) The following document is mentioned for the first time in this written opinion; the numbering will be adhered to in the rest of the procedure:

D1 : International Immunology, vol. 5, no. 8, pages
869-875

- 2) The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1 and 10 to 15 is not new in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

D1 discloses the preparation of 9 mutants of SPE-A, each with a single amino-acid substitution and the preparation of a mutant created by deletion of the 10 N-terminal amino acids. Several mutations lead to a loss of function. The mutants are expressed as fusion proteins (see "Methods on page 870). D1 also discloses th

WRITTEN OPINION

injection of one of the mutants into mice. It is disclosed that the serum of these mice is capable of specifically inhibiting the mitogenicity of SPE-A (see page 874, left hand column, second paragraph). Moreover, the description contains no evidences to show that the mutants of D1 do not possess any of the characteristics listed in present claim 10.

Moreover, it should be noted that claim 1, in its present form, is anticipated by any existing protein that is nonlethal as there is no upper limit concerning the number of aminoacid changes. This also applies to the DNA sequence according to claim 13.

- 3) As acknowledged by the Applicant on pages 1-3, the role of SPE-A in toxic shock is well established in the art. The use of the mutants disclosed in D1 to reduce symptoms associated with toxic shock is therefore obvious for the skilled person.

Claim 16 therefore does not satisfy the criterions set forth in Article 33(3) PCT.

- 4) Given that substitution of more than one amino acid does not appear to lead to any new and unexpected effects in comparison to that observed with the single substitutions carried out in D1, the subject-matter of claims 2-9 is not considered to involve an inventive step over D1. The said claims thus do not satisfy the criterions set forth in Article 33(3) PCT.
- 5) For the assessment of present claims 15 and 16 on the question as to whether they are industrially applicable, no unified criteria exist in the PCT. The patentability
-

WRITTEN OPINION

can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

To meet the requirements of Rule 5.1(a)(ii) PCT, the document D1 should be identified in the description and the relevant background art disclosed therein should be briefly discussed.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

- 1) The subject-matter of claim 1 does not enjoy substantial support in the description over the whole scope of the claims. In order to meet the requirements of Article 6 PCT, the said claim should be clarified by limitation to what is specifically and substantially disclosed in the description, i.e. the subject-matter of claim 4.
- 2) Moreover, the expression "fragment thereof" in claim 1 can be interpreted in so many ways that it renders the scope of the claim unclear, contrary to Article 6 PCT. As regards the function of the claimed mutant, it cannot be understood how a mutant can be "substantially" nonlethal. Clarification is therefore also necessary (Article 6 PCT).